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# Spectrophotometric Determination of Furosemide in Pharmaceuticals Using Permanganate

**Kalsang Tharpa, Kanakapura Basavaiah* and Kanakapura Basavaiah Vinay**

Department of Chemistry, University of Mysore, Manasagangothri, Mysore-570006, India.

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## Abstract

Based on the reduction of permanganate by furosemide (FUR) in either acidic or basic medium, two simple, sensitive and cost-effective methods were proposed for the determination of FUR in bulk drug and in formulations. In method A, FUR was treated with a measured excess of permanganate in acid medium and the unreacted oxidant was measured at 550 nm, whereas in method B the reaction was carried out in alkaline medium and the resulting manganate was measured at 610 nm. In method A, the amount of permanganate reacted corresponds to the FUR content and the absorbance was found to decrease linearly with the concentration; while in method B, the absorbance increases with concentration. Under optimum conditions, working ranges was 3.0-24.0 μg $ml^{-1}$ and 1.25-20.0 μg $ml^{-1}$ by method A and method B, respectively. The calculated molar absorptivities are 9.06 × $10^3$ and 1.36 × $10^4$ L $mol^{-1}$ $cm^{-1}$ for method A and method B, respectively, with corresponding Sandell sensitivity values of 0.0365 and 0.0243 μg $cm^{-2}$. The limits of detection (LOD) and quantification (LOQ) have also been reported. Accuracy and precision for the assay were determined by calculating the intra-day and inter-day at three concentration levels; the intra-day RSD was < 2.7% and the accuracy was better than 2.8% (RE). The methods were successfully applied to the determination of FUR in tablets and injections dosage forms; either in monopreparations of FUR or in combination with amiloride HCl. The results tallied well with the label claim and were statistically compared with those of a reference method by applying the Student's t-test and F-test. The accuracy was further ascertained from placebo and synthetic mixture analysis and also from spike-recovery method.

**Keywords:** Furosemide determination; Spectrophotometry; Potassium permanganate; Tablets.

## Introduction

Furosemide (FUR), chemically known as 5-(aminosulfonyl)-4-chloro-2-[(2-furanylmethyl) amino] benzoic acid, is structurally a sulfonamide, an antibacterial agent (Figure 1). However, FUR is a potent diuretic widely used in the treatment of edematous states associated with cardiac chronic renal failure [1,2], hypertension, congestive heart failure [3,4], and cirrhosis of the liver [5]. The official methods for the determination of FUR in dosage forms are based on titrimetry [6], spectrophotometry [7] and HPLC [8]. Besides, there are number of other techniques available in the literature and include, derivative UV spectrophotometry [9], spectrofluoremetry [10-12], HPLC with

* Corresponding author: E-mail: *basavaiahk@yahoo.co.in*; Fax: 0091-821-2421263, 2516133

UV detection [13], HPLC with fluorescence detection [14, 15], HPLC with amperometric detection [16], HPLC-DAD [17], LC-MS [18], GC-MS [19], micellar-LC [20], ratio-spectra derivative spectroscopy [21] and diffuse reflectance spectroscopy [22]., Because of its inherent simplicity, sensitivity and cost-effectiveness, visible spectrophotometry is a technique of choice employed in quality control laboratories of many developing countries. Therefore, developing a selective and sensitive methods using visible spectrophotometry is of paramount importance. Quite a few visible spectrophotmetric methods [23-34] have been developed for the quantification of FUR in pharmaceuticals which suffer from one or more disadvantage such as critical optimum conditions, heating and/or extraction step, narrow linear dynamic range and/or low sensitivity and poor selectivity. Reactions proposed for the visible spectrophotometric determination of FUR includes-reaction between FUR and $FeCl_3$ in pH range 5.2-6.2 ($\lambda_{max}$=513 nm)[23], FUR and Cu(II) at pH 3.2 using Mclivaine buffer ($\lambda_{max}$=790 nm)[24], flow injection analysis based on reduction of $KMnO_4$[25], complexation reaction with Fe(III) in ethanolic medium ($\lambda_{max}$=513 nm)[26], unreacted $Br_2$ measured after reacting with FUR ($\lambda_{max}$= 520 nm)[27], reaction between FUR and 1, 2-napthaquinone-4-sulphonate at pH 7.5 on heating for 30 min at 70 $^0$C and extracted into isoamyl alcohol[28], FUR-Pd (II) complex at pH 5.0 at 55 $^0$C ($\lambda_{max}$=410 nm)[29], FUR-iron (III) complex ($\lambda_{max}$=386 nm)[30], diazotization reaction[31], reaction with MBTH in the presence of various oxidizing agents[32], based on the reduction of paramolybdate anoin or molybdatophosphoric anion ($\lambda_{max}$=690 nm and 700 nm)[33], reaction between FUR and p-N,N-dimethylphenylenediamine dihydrochloride in the presence of chloramie-T ($\lambda_{max}$=540 nm)[34].

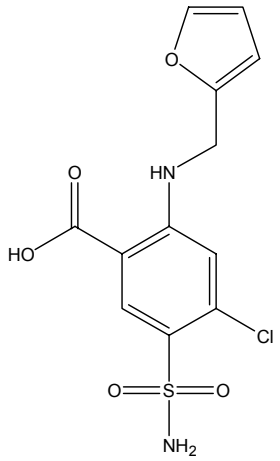


**Figure 1:** Structure of furosemide

The present paper describes two visible spectrophotometric methods based on the reduction of $KMnO_4$ in acid and basic mediums. Simplicity, sensitivity, wide linear ranges, mild experimental conditions and above all cost-effectiveness characterize the proposed methods. Further, the methods were found to possess adequate accuracy and precision.

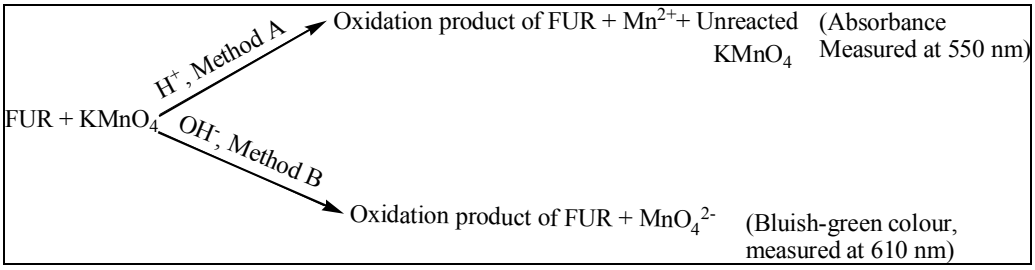


**Figure 2:** Tentative reaction scheme for method A and method B.

## Experimental

*Apparatus*

A Systronics model 106 digital spectrophotometric with 1-cm matched quartz cells was used for all absorbance measurements.

*Reagents and Standards*

All chemicals used were of analytical reagent grade and distilled water was used to prepare all solutions. Potassium permanganate ($1\times10^{-2}$ M) was prepared by dissolving 0.395 g of the chemical (Merck, Mumbai, India) in water, the solution was boiled for 10 min to remove any residual manganese (IV) ions, cooled, filtered and diluted to 250 ml and standardized using H.A Bright's procedure [35]. It was diluted to get 500 µg $ml^{-1}$ for method A and 2 mg $ml^{-1}$ for method B. Acetic acid (3:2, v/v) was prepared by diluting glacial acetic acid (Merck, Mumbai, India, Sp. gr. 1.05) appropriately with water. Sodium hydroxide solution (0.1 N) was prepared by dissolving the chemical (Merck, Mumbai, India) in water. Pharmaceutical grade FUR, certified to be 99.85% pure, was kindly provided by Hoechest Morrison Roussel Ltd., Mumbai, India, as gift and was used as received. For method A, 60 µg $ml^{-1}$ FUR was prepared by dissolving 15.0 mg of FUR in 3:2 acetic acid and made up to 250 ml with the same acid. While for method B, 50 µg $ml^{-1}$ FUR was prepared by dissolving 12.5 mg of FUR in 0.1 N NaOH and made up to 250 ml with 0.1 N NaOH. Tablets and injections containing FUR alone or in combination with amiloride HCl were purchased from local market.

*Method A*

Different aliquots of standard solution (0.5-4.0 ml, 60 µg $ml^{-1}$) of pure FUR prepared in acetic acid were transferred into a series of 10 ml volumetric flasks by means of micro burette and the total volume was adjusted to 4.0 ml with 3:2 acetic acid. To each flask, 1 ml of 5 M $H_2SO_4$ was added followed by 1 ml of 500 µg $ml^{-1}$ $KMnO_4$, the latter being measured accurately. The flasks were kept aside for 10 min with occasional shaking before diluting to the mark with water. The absorbance was recorded at 550 nm against water blank.

*Method B*

Into a series of 10 ml volumetric flasks, 0.25-4.0 ml of 50 µg $ml^{-1}$ pure FUR solution made in NaOH were added by means of micro burette and the total volume was made up to 4.0 ml with 0.1 N NaOH. To each flask, 1 ml of 2 mg $ml^{-1}$ $KMnO_4$ solution was added. The flasks were kept aside for 15 min with occasional shaking and the volume was made up to the mark with water. The absorbance was recorded at 610 nm against the reagent blank.

*Procedure for Tablets/Injection/Combination tablets*

Twenty tablets containing FUR alone or twenty tablets containing FUR in combination with amiloride HCl were separately weighed and ground into fine powder (and preserved separately in two amber-colored bottles). An amount of either powder equivalent to 30 mg of FUR was weighed into a 50 ml volumetric flask, 30 ml of glacial acetic acid was added and the mixture was shaken for 20 min; the volume was then made up to the mark with water, mixed well and filtered using Whatman No. 42 filter paper. The filtrate equivalent to 600 µg $ml^{-1}$ FUR was diluted to 60 µg $ml^{-1}$ concentration and a convenient aliquot was subjected to analysis using the procedure described under method A. Another portion of either tablet powder equivalent to 25 mg of FUR was weighed into a 50 ml volumetric flask, 30 ml of acetone was added and the mixture was shaken for 5 min. The mixture was filtered using Whatman No. 42 filter paper and the filtrate was evaporated to dryness on a water bath. The residue was washed thoroughly several times with water before dissolving it in 0.1 N NaOH. The solution was then transferred into a 50 ml volumetric flask, made up to the mark with 0.1 N NaOH and suitable aliquot was then subjected to analysis using the procedure described under method B, after diluting to 50 µg $ml^{-1}$ solution. Contents of twenty ampoules each containing 10 mg of FUR were pooled and mixed to get a homogeneous solution. An aliquot equivalent to 60 mg of FUR was accurately measured and transferred into previously dried 100 ml calibration flasks and made up to the mark with (3:2) acetic acid for use in method A. The solutions were diluted appropriately to achieve working concentration of 60 µg $ml^{-1}$ FUR for method A and analyzed using the procedures described earlier. Analysis of FUR in injection by method B was unsuccessful since some diluents which were present interfered seriously with the analysis procedure and could not be removed by extraction with acetone.

## Results and Discussions

Although permanganate is a strong oxidizing agent that can react with several organic substances, the tablet excipients in the analyzed samples did not interfere in case of method A and the interference due to excipients in the case of method B was successfully overcome by extraction with acetone. Recently, permanganate was studied to determine pharmaceutical active compounds in formulations both in acidic medium [12, 36] as well as in alkaline medium [37-40]. The titration of FUR (2.0-9.0 mg) against 0.03 M $KMnO_4$ in 5 mL of 5M $H_2SO_4$ medium followed a 1:4 reaction

stiochiometry (FUR: $KMnO_4$) (The work has been communicated to *Proceedings National Academy of Science, New Delhi, India*). The studies of Drummond and Waters [41] showed that the reduction of permanaganate involves one electron change in alkaline medium forming bluish green manganate, which served as the chromogen for assay in method B.

Optimisation *of Experimental Conditions*

In method A, when a fixed concentration of permanganate was reacted with increasing concentrations of FUR in $H_2SO_4$ acid medium, a concomitant fall in the concentration of permanganate occurred as revealed by the decreasing absorbance at 550 nm (Figure 3 & Figure 4), which served as the basis for quantification. A preliminary experiment showed that permanganate can be determined up to 50 µg $ml^{-1}$ (Figure 5) at 550 nm under the optimum acidic conditions of assay. Hence, different concentrations of FUR were reacted with 1 ml of 500 µg $ml^{-1}$ $KMnO_4$ to determine the concentration range over which FUR could be determined. To check the effect of acid concentration on the reaction, 0-5 ml of 5 M $H_2SO_4$ was added to the fixed concentration of FUR and $KMnO_4$. It was observed that there was absolutely no change in the absorbance when 1-5 ml of 5 M $H_2SO_4$ were used in a total volume of 10 ml. Effect of hydrochloric acid was not studied since $KMnO_4$ being a strong oxidizing agent would react with HCl to liberate chlorine. The reaction between FUR and $KMnO_4$ in the acid concentration employed was complete in 10 min (Figure 6), and the absorbance of the measured unreacted $KMnO_4$ was found to be stable up to 40 min thereafter. Appreciable change in the absorbance after 40 min could be due to the slow reaction between excess $MnO_4^-$ with relatively high concentration of $Mn^{2+}$. Two blanks were prepared for the study. Blank-1: Consist of 4.0 ml of 3:2 acetic acid, 1 ml of 5M $H_2SO_4$ and 1 ml of 500 µg $ml^{-1}$ $KMnO_4$ in a total volume of 10 ml (adjusted by water). This shows maximum absorbance reading at 550 nm against water. Blank-2: Consist of 4.0 ml of 3:2 acetic acid and 1 ml of 5M $H_2SO_4$ in a total volume of 10 ml (adjusted by water) shows negligible absorbance reading. Hence, all readings are taken against water.

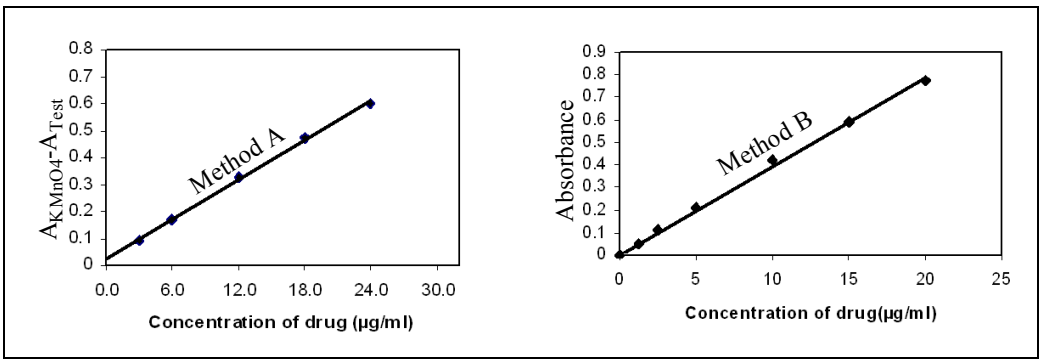


**Figure 3:** Calibration graphs for method A and method B.

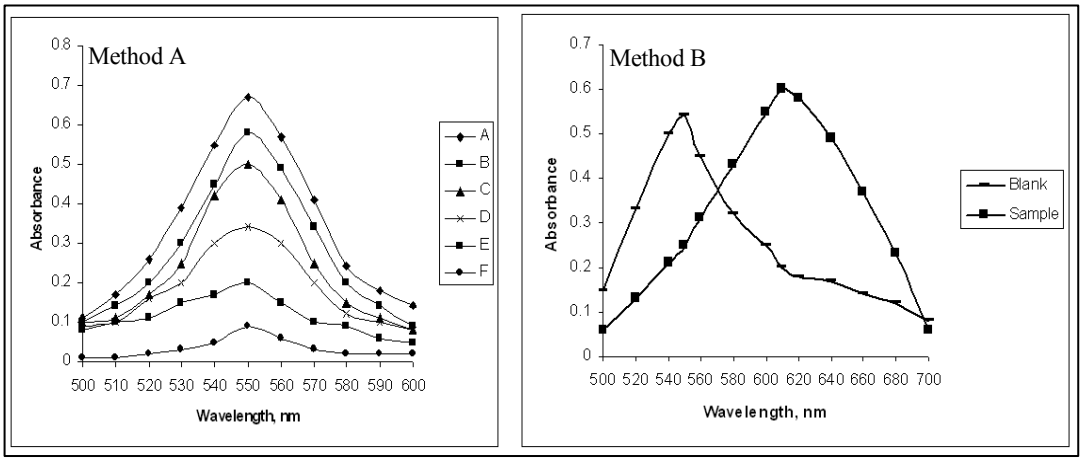


**Figure 4:** Absorption spectra for method A and method B

Method A: (A.0.0; B.3.0; C.6.0; D.12.0; E.18.0; F.24.0 µg $ml^{-1}$ FUR).
Method B: (Bluish green color produced for 15 µg $ml^{-1}$ FUR).
Blank: Absorbance against water.

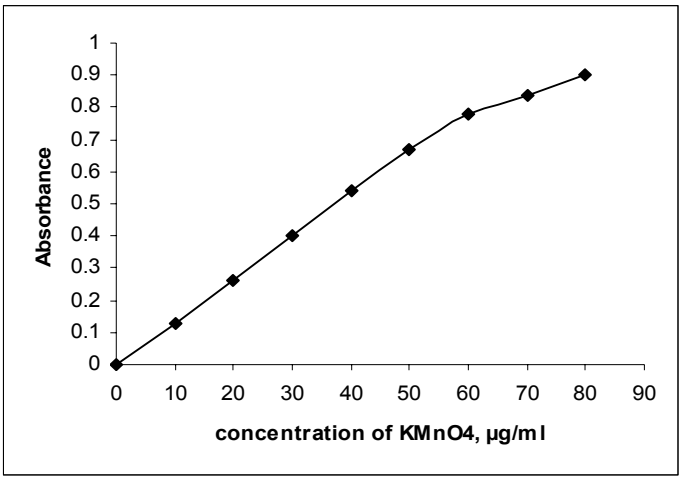


**Figure 5:** Linear relation between absorbance at 550 nm and $KMnO_4$ in 0.5M $H_2SO_4$.

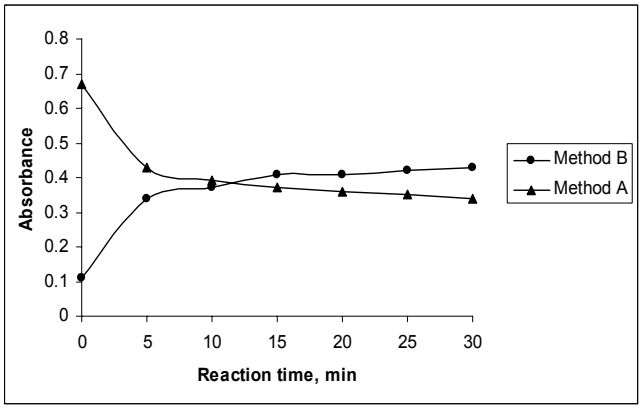


**Figure 6:** Effect of reaction time in method A and method B.

Potassium permanganate quantitatively oxidizes FUR in the presence of NaOH in method B, resulting in the formation of bluish-green color of manganate ion which

showed absorption peak at 610 nm (Figure 4) and served as the basis for the calibration graph (Figure 3). An increase in the concentration of $KMnO_4$ could enhance sensitivity of the method but the blank absorbance also increased concomitantly. The effect of $KMnO_4$ concentration on the sensitivity of the reaction (Figure 7) based on which the optimum concentration was fixed at 0.2 mg $ml^{-1}$ (1 ml of 2 mg $ml^{-1}$ in a total volume of 10 ml). The overall NaOH concentration employed (0.04 N) is not critical, since higher concentrations did not affect either the sensitivity or stability of the reaction product. The reaction was complete in 10 min (Figure 6) and the contact time is not critical and any delay up to 30 min had no effect on the absorbance. The absorbance of the measured color was constant for 50 min in the presence of unreacted $KMnO_4$ and the reaction product. Reagent blank consists of 4.0 ml of 0.1 N NaOH and 1 ml of 2mg $ml^{-1}$ $KMnO_4$ in a total volume of 10 ml (adjusted by water). This shows absorbance reading of 0.15 against water. Hence, all readings were taken against reagent blank.

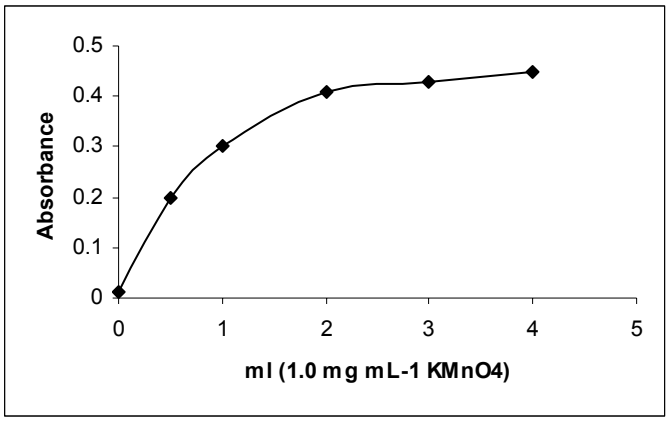


**Figure 7:** Effect of $KMnO_4$ concentration in method B.

Analytical *data*

Calibration graphs for method A and method B are described by the regression equation:

Y = 0.0249 + 0.0244 X, for method A and

Y = 0.0243 + 0.0377 X, for method B

(Where Y = absorbance of 1-cm layer of solution and X = concentration in µg $ml^{-1}$). In method A, the different between the reagent blank absorbance and the sample absorbance was plotted *vs* the FUR concentration. The limits of detection (LOD) and quantitation (LOQ) calculated according to ICH guidelines [42] were 0.74, 0.36 and 2.25, 1.10, respectively for method A and method B. The significance of correlation coefficients in method A and method B was evaluated by calculating the t-values using the following formula [43].

$$t = \frac{|r|\sqrt{n-2}}{\sqrt{1-r^2}}$$

The calculated values were found to be 60.24 and 76.18 for method A and method B, respectively. Thus, it can be concluded that there is significant relationship between the concentration of FUR and the variables used in the development of regression equations.

*Method Validation*

*Assay precision and accuracy*

The precision of the methods was calculated in terms of intermediate precision (intra-day and inter-day) [44]. Three different concentrations of FUR were analysed in seven replicates during the same day (intra-day precision) and five consecutive days (inter-day precision). The RSD (%) values of intra-day and inter-day studies showed that the precision was good (Table 1). The accuracy of an analytical method expresses the closeness between the reference value and the found value. Accuracy was evaluated as percentage relative error between the measured concentrations and nominal concentrations for FUR (Bias %). The results obtained are compiled in table 1 and show that the accuracy is good.

**Table 1.** Intra-day and Inter-day precision and accuracy evaluation

| Method | FUR µg $ml^{-1}$ taken | Intra-day (n=7) | | | Inter-day (n=5) | | |
|---|---|---|---|---|---|---|---|
| | | FUR µg $ml^{-1}$ found[a] | Precision[b] | Accuracy[c] | FUR µg $ml^{-1}$ found[a] | Precision[b] | Accuracy[c] |
| A | 6.000 | 6.140 | 2.580 | 2.330 | 6.150 | 3.160 | 2.500 |
| | 12.00 | 11.72 | 2.660 | -2.330 | 12.33 | 2.870 | 2.750 |
| | 18.00 | 17.82 | 1.960 | -1.010 | 18.27 | 2.420 | 1.500 |
| B | 5.000 | 5.10 | 2.630 | 1.960 | 5.110 | 3.020 | 2.200 |
| | 10.00 | 10.19 | 2.180 | 1.900 | 10.25 | 2.360 | 2.500 |
| | 15.00 | 15.12 | 1.310 | 0.840 | 15.20 | 2.180 | 1.340 |

a. Mean ± standard error, b. Relative standard deviation (%), c. Bias %: (found-taken/taken) x 100.

*Method Selectivity*

Method selectivity was evaluated by preparing a synthetic mixture and it was confirmed that the change in signal measured (absorbance) was caused only by the analyte. A synthetic mixture consisting of 20 mg sodium alginate, 30 mg magnesium stearate, 20 mg lactose, 20 mg acacia, 50 mg talc and 30 mg starch besides 20 mg of FUR was prepared and analysed after extraction into acetic acid in method A and into acetone in the case of method B as described under “procedure for tablets/injection/combination tablet”. The percent recoveries of FUR were 101 ± 0.86 and 98.58 ± 0.63 for method A and method B, respectively. This confirms the selectivity of methods under the optimized conditions.

*Matrix effect*

Matrix effect was carried out in order to find the interference. A placebo blank consisting of 20 mg sodium alginate, 30 mg magnesium stearate, 20 mg lactose, 20

mg acacia, 50 mg talc and 30 mg starch but without FUR was prepared and analysed as described under “procedure for tablets/injection/combination tablet”. There was absolutely no interference from the placebo in method A but huge interference was encountered in method B. The interference from placebo mixture in method B was successfully overcome by extraction of FUR into acetone and performing the analysis as described under “Procedure for Tablets/Injection/Combination tablet”.

*Application to analysis of pharmaceutical formulations*

Method A doesn’t suffer from interference from the tablet excipients and results in Table 2 show close agreement between the results obtained by the proposed methods and the label claim. Method B entails extraction of FUR into acetone since there was some interference from the excipients when applied directly to the tablet extract in NaOH. The acetone was later evaporated and the residue was dissolved in NaOH after repeatedly washing with water and an appropriate working concentration of FUR was prepared and analyzed as given under “procedure for tablets/injection/combination tablet”. FUR in injection could be determined without interference using method A. However, interference due to co-formulated substances in injections could not be overcome in method B by simple extraction with acetone as done for tablets. The results were compared statistically by applying Student’s t-test for accuracy and variance ratio F-test for precision with those of the literature method [7] at 95% confidence level. The literature method consisted of extraction of FUR from the matrices using 0.1N NaOH and detection at 271 nm. The calculated t-test and F-values (Table 2) did not exceed the tabulated values of 2.78 and 6.39, respectively, indicating no significant difference between the proposed methods and the reference method in terms of accuracy and precision. The validity of the methods was confirmed by applying the standard addition technique. Pre-analyzed tablet powder containing FUR was spiked with pure FUR at three concentration levels and the total was measured by the proposed methods. Each determination was done three times. The results of this study are compiled in table 3.

**Table 2:** Summary of furosemide assay results for different dosage forms

| Tablets/Injection/ Combination tablet analyzed | Label claim | Found* (Percent of label claim ±SD) | | |
|---|---|---|---|---|
| | | Reference method | Method A | Method B |
| Frunex[a] (FUR alone) | 100 mg/Tab | 95.84 ± 1.26 | 94.50 ± 2.48<br>**t=** 1.13<br>**F=** 3.87 | 96.12 ± 2.64<br>**t=** 0.22<br>**F=** 4.39 |
| Lasix[b] (FUR alone) | 40 mg/Tab | 99.32 ± 1.08 | 98.46 ± 2.32<br>**t=** 0.79<br>**F=** 4.61 | 101.10 ± 1.74<br>**t=** 1.88<br>**F=** 2.59 |
| Lasix[c] (Injection) | 10 mg/Ampoule | 101.9 ± 1.15 | 102.3 ± 1.42<br>**t=** 0.49<br>**F=** 1.52 | ------ |
| Amifru[d] (Combination Tab) | 40 mg/Tab | 104.0 ± 1.36 | 103.6 ± 2.58<br>**t=** 0.32<br>**F=** 3.59 | 103.9 ± 2.75<br>**t=** 0.08<br>**F=** 4.08 |

*Mean value of five determinations

Marketed by:
[a]Geno Pharmaceuticals Ltd. Karaswada, Goa-403507
[b]Aventis Pharma Ltd. Ankleshwar-393002
[c]Aventis Pharma Ltd. Thane-401506
[d] Elder Pharmaceuticals Pvt Ltd. Mumbai-400053
Tabulated t-value at the 95% confidence level is 2.78; Tabulated F-value at the 95% confidence level is 6.39.

**Table 3:** Results of recovery study by standard addition method

| Formulation studied | Method A | | | | Method B | | | |
|---|---|---|---|---|---|---|---|---|
| | FUR in tablet, µg $ml^{-1}$ | Pure FUR added, µg $ml^{-1}$ | Total found, µg $ml^{-1}$ | Pure FUR recovered*, Percent ± SD | FUR in tablet, µg $ml^{-1}$ | Pure FUR added, µg $ml^{-1}$ | Total found, µg $ml^{-1}$ | Pure FUR recovered*, Percent ± SD |
| Lasix 40 mg | 7.880 | 4.000 | 11.77 | 97.25 ± 2.17 | 8.090 | 4.000 | 12.26 | 104.30 ± 1.85 |
| | 7.880 | 8.000 | 15.93 | 100.60 ± 1.78 | 8.090 | 8.000 | 16.59 | 106.2 ± 2.67 |
| | 7.880 | 12.00 | 20.28 | 103.30 ± 2.36 | 8.090 | 12.00 | 20.27 | 101.5 ± 2.19 |
| Amifru 40 mg combination Tablet | 8.290 | 4.000 | 12.36 | 101.70 ± 1.62 | 8.310 | 4.000 | 12.32 | 100.3 ± 2.48 |
| | 8.290 | 8.000 | 16.81 | 106.50 ± 2.46 | 8.310 | 8.000 | 16.59 | 103.5 ± 1.84 |
| | 8.290 | 12.00 | 20.85 | 104.70 ± 2.75 | 8.310 | 12.00 | 20.51 | 101.7 ± 2.34 |

*Mean value of three determinations

## Conclusion

The proposed two methods are free from rigid experimental conditions and are characterized by wide linear dynamic ranges and high sensitivity, and employ inexpensive and easily available chemicals and hence cost-effective when compared to the existing spectrophotometric methods. Method A is simpler and can be extended successfully to the quantification of FUR present in the injections and combination tablets without interference from the other active ingredients. However, method B entails an extraction step when applied to tablets to overcome the interference from some inactive ingredients. The low detection and quantification limits, simplicity and selectivity make the method suitable for the quality control in the pharmaceutical industry for routine analysis.

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